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TOPICAL REVIEW

Silicon central pattern generators for cardiac diseases

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Abstract Cardiac rhythm management devices provide therapies for both arrhythmias and resynchronisation but not heart failure, which affects millions of patients worldwide. This paper reviews recent advances in biophysics and mathematical engineering that provide a novel technological platform for addressing heart disease and enabling beat-to-beat adaptation of cardiac pacing in response to physiological feedback. The technology consists of silicon hardware central pattern generators (hCPGs) that may be trained to emulate accurately the dynamical response of biological central pattern generators (bCPGs). We discuss the limitations of present CPGs and appraise the advantages of analog over digital circuits for application in bioelectronic medicine. To test the system, we have focused on the cardio-respiratory oscillators in the medulla oblongata that modulate heart rate in phase with respiration to induce respiratory sinus arrhythmia (RSA). We describe here a novel, scalable hCPG comprising physiologically realistic (Hodgkin–Huxley type) neurones and synapses. Our hCPG comprises two neurones that antagonise each other to provide rhythmic motor drive to the vagus nerve to slow the heart. We show how recent advances in modelling allow the motor output to adapt to physiological feedback such as respiration. In rats, we report on the restoration of RSA using an hCPG that receives diaphragmatic electromyography input and use it to stimulate the vagus nerve at specific time points of the respiratory cycle to slow the heart rate. We have validated the adaptation of stimulation to alterations in respiratory rate. We demonstrate that the hCPG is tuneable in terms of the depth and timing of the RSA relative to respiratory phase. These pioneering studies will now permit an analysis of the physiological role of RSA as well as its any potential therapeutic use in cardiac disease.

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Abbreviations ADC, analog-to-digital converter; bCPG, biological central pattern generator; BötC, Bötzinger complex; CPG, central pattern generator; CPU, central processing unit; CRT, cardiac resynchronisation therapy; cVN, central vagus nerve; DAC, digital-to-analog converter; hCPG, hardware central pattern generator; HR, heart rate; GPN, glossopharyngeal nerve; LBBB, left bundle branch block; NHS, National Health Service (UK); PN, phrenic nerve; r.m.s, root mean square; RSA, respiratory sinus arrhythmia; RTN, retro-trapezoid nucleus; RVLM, rostral ventro lateral medulla; sCPG, software central pattern generator; SN, sympathetic nerve; τ_{AV}, atrioventricular delay; τ_{VV}, interventricular delay; VN, vagus nerve; VRG, ventral respiratory group.

Alain Nogaret is a physicist with interest in building and studying artificial neurons and networks that make constructive use of the principles of nonlinear science to develop novel therapies for chronic diseases. He has demonstrated enhanced transmission of electric pulses by stochastic resonance in semiconductor neurons that have dendrites, soma and axon compartments. He has also demonstrated the coexistence of multiple motor patterns in central pattern generator hardware and their activation by command neuron signals. **Erin O'Callaghan**, **Renata Lataro** (research fellows) and **Julian Paton** are all experimental physiologists interested in neural control systems of arterial pressure and respiration.

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An unmet clinical need

Heart failure affects around 900,000 people in the UK, accounts for 5% of emergency hospital admissions and utilises 2% of all NHS hospital bed days (Cleland *et al.* 2012). In the USA, over 1 million patients are admitted to hospital for congestive heart failure (Hall *et al.* 2012). Despite use of the optimal medical therapy available, the prognosis remains poor for this group of patients who also have a high symptom burden. Patients in whom symptoms are refractory to medical therapy may be considered for cardiac resynchronisation therapy (CRT) using biventricular pacemakers. Unfortunately, these devices are only appropriate in a minority of patients with a left bundle branch block pattern (LBBB) ECG (25% of the total heart failure population). Furthermore, amongst recipients of CRT, 30% of patients do not respond clinically (Jeevanathan *et al.* 2009). Thus, there remains a substantial unmet clinical need in the heart failure population and new interventional therapies are required. An early prognosis indicator of heart disease is loss of respiratory sinus arrhythmia and this raises the question as to its physiological role and whether reinstatement could provide therapeutic benefit.

Respiratory sinus arrhythmia (RSA) and its loss in cardiovascular disease

The healthy heart is subjected to chronotropic modulation by the respiratory cycle (Anrep *et al.* 1936); this is respiratory sinus arrhythmia (RSA). RSA is a naturally occurring arrhythmia that is prominent at birth and in athletes but is lost with ageing and cardiovascular disease. RSA is brought about by a combination of afferent feedback (pulmonary lung inflation, baroreceptors) and central coupling between medullary respiratory and cardiac vagal motor neurones (McAllen & Spyer, 1978; McAllen *et al.* 2011). Despite RSA being highly preserved during evolution (cartilaginous fish, amphibians, reptiles) its functional significance remains both incompletely understood and controversial (Larsen *et al.* 2010). It has been suggested that the physiological function of RSA is to match ventilation and perfusion in the lungs thereby optimising oxygen uptake and carbon dioxide removal (Hayano *et al.* 1996). In dogs, Giardino *et al.* (2002) showed that dead space-to-tidal volume ratio and intrapulmonary shunt fraction decreased and that oxygen uptake was enhanced. They measured the ventilatory equivalents of CO_2 and O_2 during paced breathing in healthy humans and concluded that gas exchange efficiency increased with RSA. In contrast, Sin *et al.* (2010) concluded that the improvements in gas exchange efficiency were unrelated to RSA in humans. Additionally, Elstad has reported a role for RSA in stabilising blood pressures generated from the left and right ventricles (Elstad, 2012). Finally, Grossman & Taylor (2007) discussed the importance of RSA for optimising energy use by cardio-respiratory synchronisation, consistent with the idea that RSA saved heart beats.

Recently, we built a mathematical model to understand the functional role of RSA and to address the inconsistencies of previous reports (Ben-Tal *et al.* 2012, 2014). We demonstrated that although gas exchange efficiency can improve with both slow and deep breathing, and increased mean heart rate, this was all unrelated to RSA. Rather, we showed that RSA minimises the work done by the heart, so saving energy and making it more efficient, while maintaining physiological levels of arterial carbon dioxide. Given that poor control of arterial CO_2 (hypocapnia) contributes to central sleep apnoea in ∼45% of patients with heart failure (Javaheri *et al.* 1995), RSA may, via its ability to regulate P_{aCO_2}, reduce incidence of central sleep apnoea and provide therapeutic benefit for a failing heart. Consistent with this notion is the finding that RSA may increase cardiac efficiency by saving energy (Ben-Tal *et al.* 2012, 2014). This result has led to the notion that in heart failure, a condition when heart rate variability is diminished, re-instatement of RSA could provide therapeutic benefit to cardiac pumping (Mortara *et al.* 1994). To begin to address this, we have designed and tested a silicon hardware central pattern generator (hCPG) based on realistic neurones with Hodgkin–Huxley kinetics.

Why biological central pattern generators (bCPGs) are better than digital electronics for rhythm generation

The study of bCPGs of invertebrates (Kristan *et al.* 2005; Marder *et al.* 2005; Selverston, 2009) has shown why neural networks are better suited than digital electronics for generating biological rhythms. First, neurons are intrinsically non-linear devices. Non-linearity, which is often an undesirable property in conventional electronic design, is the property that enables bCPGs to integrate their motor output based on incoming biological stimuli (Rabinovich *et al.* 2000). Second, bCPGs are able to maintain the precise timing of burst discharges within the rhythmic cycle by adapting neuron phase lag to changes in rhythm frequency. Marder *et al.* (2005) have ascribed this property to frequency-dependent synaptic depression or activation of transient outward potassium current on release from a hyperpolarised membrane potential, for example. These mechanisms are essential for maintaining the correct sequence of motor signals as the rhythmic frequency increases (Mamiya & Nadim, 2004). For instance, the neuromeres of leech maintain the sequence of contraction of body wall segments as the swimming rate increases (Kristan *et al.* 2005). This approach could conceivably be used to maintain the contraction of

the four chambers of the heart during tachycardia, for example. However, existing cardiac resynchronisation pacemakers have fixed timings which are adjusted by the practitioner and cannot adapt when heart rate increases. An hCPG, however, would provide beat-to-beat instantaneous adjustment of the inter-ventricular (τ_{VV}) and atrio-ventricular (τ_{AV}) delays.

The basic building block of a bCPG is the half-oscillator which consists of two neurons inhibiting each other (Varona *et al.* 2001). This elementary circuit has been thoroughly studied in the leech where it acts as a two phase cardiac pacemaker (De Schutter *et al.* 1993; Marder & Calabrese, 1996) producing fundamental heartbeat. The inhibitory pair is surrounded by four pairs of inter-neurons whose role is to switch motor drive between the two hearts of the leech in cycles lasting 20–40 beats (Norris *et al.* 2006). Three-phase bCPGs are found in the stomatogastric ganglion of the lobster and the respiratory pattern generator of *Lymnaea* (Taylor & Lukowiak, 2000; Selverston, 2009). They incorporate a third neuron which interacts with the others via mutually inhibitory links. In mammals, respiratory rhythms are equally generated by a three phase bCPG located in the brainstem (Fig. 1). This consists of three neurons: early-I (early inspiration), post-I (post inspiration) and aug-E (late inspiration) (Richter, 1982; Smith *et al.* 2007; Abdala *et al.* 2009). These neurones are considered by us to be connected together via reciprocal inhibitory synapses (Smith *et al.* 2007, 2013). It has been argued that the coupled respiratory oscillators, which are active in the expiratory phase and located in the Bötzinger complex (BötC: post-I and aug-E neurons) and in the inspiratory phase in the pre-Bötzinger complex (pre-BötC: early-I), derive from the gill and lung oscillators of earliest air breathers (Vasilakos *et al.* 2005). Neurocircuitry in the ventrolateral medulla oblongata couples the respiratory oscillator with both cardiac vagal preganglionic motoneurones, which slow the heart rate in expiration, and the diaphragm, to inflate lungs (Fig. 1). The amplitude and phase of heart rate modulation depend on physiological feedback (blood pressure, lung inflation, hormonal release) which is integrated, in part, by the circuit in Fig 1. It is this function that we would ideally wish to replicate to either boost or restore naturally occurring RSA.

Generalising this to networks of N neurons reciprocally connected by inhibitory synapses gives so-called Winnerless dynamics (Laurent *et al.* 2001). As neurons compete for firing, electrical activity 'bounces' from one neuron to another along closed loop trajectories producing competition without a winner (Rabinovich *et al.* 2001, 2006). Theoretical studies of these inhibitory networks have demonstrated two more properties of CPGs. The firing sequence of neurons along these trajectories generates motor patterns which are extremely stable with respect to noise yet very sensitive to changes in input signal. The reason for this is that neurons that compete via mutually inhibitory connections develop mildly chaotic dynamics characterised by attractors surrounded by basins of attraction. Assuming moderate noise is present in the system, its effect is only to perturb the oscillations of the CPG about the attractor. The CPG then returns to the stable oscillatory mode when the perturbation has been removed. In contrast, a pulsed input, which unlike noise has non-zero time average, may kick the CPG state from one basin of attraction to another by moving across dynamic saddle points. This has the advantage that pacing devices based on neural electronics will be inherently safer because they are not easily perturbed when set into a given mode of oscillation. The CPG theoretically behaves like an associative memory with $(N-1)!$ attractors where the same circuit will produce different modes of oscillation in response to a given stimulus (Bick & Rabinovich, 2009). Lastly, CPGs are very robust. The natural life span of the lobster is 25 years and the rhythmic patterns of the stomatogastric ganglion must be preserved over this time despite changes in neurotransmitter production. This bCPG has been shown to restore the original modes of oscillation even after the structure of the network had been impaired (Thoby-Brisson & Simmers, 2002). In *Drosophila*, the connectivity of CPGs is present at birth and this enables the correct motor patterns to be generated innately (Suster & Bate, 2002). The mammalian respiratory rhythm generator is another good example of the robustness of the bCPG despite constant state changes during exertion, speech, swallowing and sleep states. The addition of bCPG-like technology to pacemakers, which are known to be affected by parasitic electromagnetic fields in the leads (e.g. within magnetic resonance scanners), would improve the safety of cardiac rhythm management. hCPGs emulating the neuro-circuitry of the brainstem (Fig. 1) have the potential to restore a number of important cardiac functions (Fig. 2). These include: RSA, adaptation of heart rate to effort and beat-to-beat adaptation of atrio-ventricular and inter-ventricular delays in cardiac resynchronisation.

The limitations of software neuron CPGs

CPGs have been modelled using computational neuron models to investigate network dynamics (Rabinovich *et al.* 2001, 2006). Computer simulations of these networks (sCPGs), however, rely on increasingly reductive neuron models as network size increases. One reason for this is that large systems of Hodgkin–Huxley equations (Hodgkin & Huxley, 1952) develop stiffness as a result of incorporating multiple time constants. The accurate integration of stiff systems of equations causes computer processing time to rise exponentially with the size of the network. Wojcik

et al. (2011) and Zhao & Nogaret (2014) have mapped the dynamics of three Hodgkin–Huxley neurons that interact through mutually inhibitory synapses. The time required for integrating the output of such an elementary network or even a single neuron with seven ion channels (Meliza *et al.* 2014) is of the order of several minutes. Digital technology is therefore inadequate for making cardiac implants that must integrate physiological feedback in *real time*. Approximate neuron models are not suitable either as neurons must produce motor signals with the same degree of realism as complex mammalian neurons, which host a wide range ion channels. In contrast, software neurons have the advantage of flexibility and have been successfully interfaced with bCPGs to substitute one bCPG neuron with a software neuron model running on digital processing board. In this way, Olypher *et al.* (2006) and Sorensen *et al.* (2004) have controlled the burst duty cycle of the leech heart bCPG by artificially changing calcium and h-current properties in the model neuron. Prinz *et al.* (2004) and Sharp *et al.* (1993) have developed computer controlled conductances that inject specific time-dependent currents in order to tune the coupling between neurons. This has enabled Pinto *et al.* (2001) to change the firing sequence of stomatogastric neurons in the lobster. To meet the demands of greater performance for neural model simulation and dynamic clamps, several groups are turning to field programmable gate arrays (Graas *et al.* 2004; Mak *et al.* 2006).

Hardware implementations – the analog way

Analog neural computation is the approach needed for generating accurate motor patterns in real time with instantaneous response time irrespective of the size of the network or the complexity of the neuron models. To date, technologists have only implemented hCPGs for engineering applications such as modelling the gait of robots (Briggman & Kristan, 2008), the walking and swimming motions of the salamander (Ijspeert *et al.* 2013) or the undulation of robotic fish (Lee *et al.* 2007). However, neurons used in these studies are simplified models based

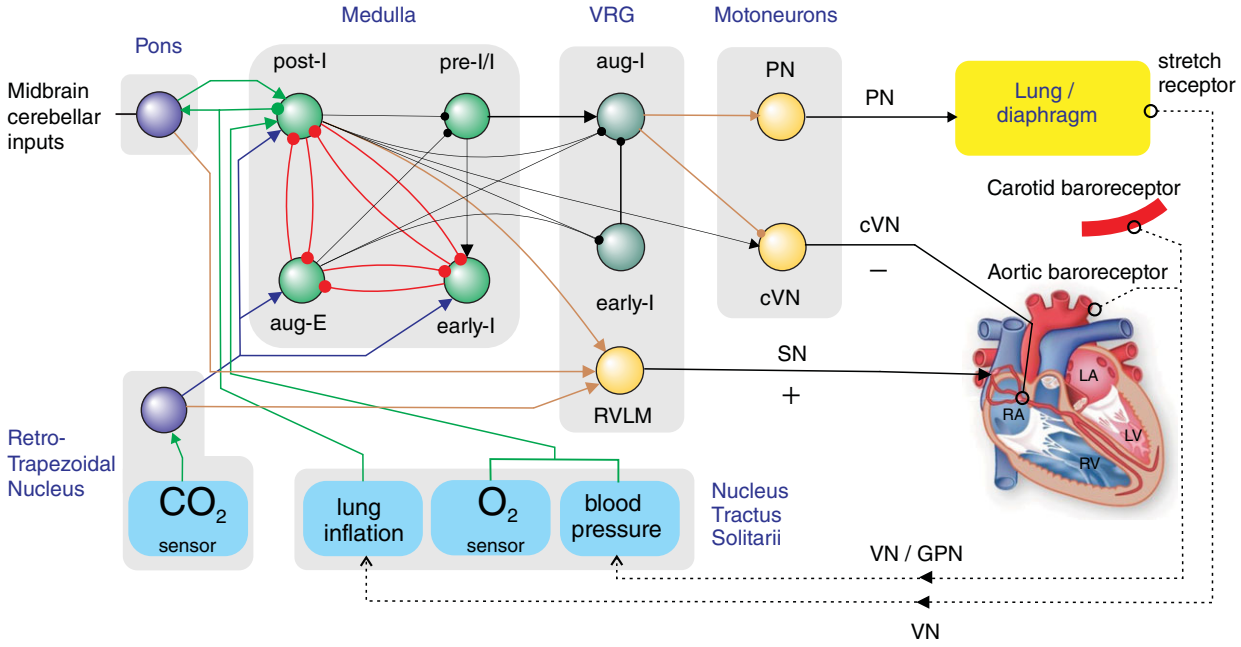

Figure 1. Regulation of the cardio-respiratory system by biological CPGs (bCPGs) in the brainstem
The respiratory bCPG is based on a group of three mutually inhibitory neurons named post-I, aug-E and early-I which generate a three phase rhythm in the following sequence: inspiration (early-I fires), post-inspiration (post-I fires) and expiration (aug-E fires). Pulmonary lung inflation stimulates vagal feedback that inhibits inspiratory neurons. By projecting excitatory synapses to post-I neurons, arterial baroreceptors provide part of the feedback responsible for respiratory sinus arrhythmia. The central coupling between post-I and cardiac vagal motoneurones (cVN) contributes to respiratory sinus arrhythmia (RSA) also. Inspiration is driven by the activation of the pre-I and early-I neurons that via aug-I neurones send signals to inhibit cVN and excite phrenic motoneurones that innervate the diaphragm. cVN activity has the effect of slowing heart rate. This depressive action is balanced by rostral ventrolateral medulla (RVLM) neurons that accelerate heart rate and increase the force of contraction via sympathetic innervation. Figure based on circuits described in Smith *et al.* 2007 and Smith *et al.* 2013. Abbreviations: VRC, ventral respiratory group; PN, phrenic nerve; cVN, cardiac vagus nerve; SN, sympathetic nerve; VN, vagus nerve; GPN, glossopharyngeal nerve; RTN, retro-trapezoid nucleus; ⟶, excitatory link; —●, inhibitory link.

on various degrees of approximation, which would be unsuitable for driving physiological systems that need to integrate afferent feedback signals *in vivo*. Lee *et al.* (2007) used the Hindmarsh–Rose neuron (Hindmarsh & Rose, 1984) which models bursting dynamics but is constrained by a stiff parameter space that cannot be easily adjusted to fit real neurons. Nakada *et al.* (2003) have proposed an hCPG that generates the motor sequences of different horse gaits: walk, trot and gallop. Switching from one gait to another, however, involved a *physical reconfiguration* of the network rather than the application of the appropriate *stimulus* to switch from one oscillator (attractor) mode to another (Zhao & Nogaret, 2014). To our knowledge hCPGs have not yet been developed for neuro-stimulation. Our interests are in building hCPGs for naturalistic and adaptive pacing of cardiac function, as described in Fig. 2.

In Table 1, we list the advantages of hCPGs for medical devices. Analog circuits are simpler than digital ones due to the elimination of analog-to-digital conversion, microprocessor and memory. Thus, they are easier to scale down on a silicon chip for implanting. They have lower power requirements that increases battery lifetime beyond the lifetime of the patient. They integrate input signals in real time and with perfect accuracy irrespective of the number of channels or compartments in the neurons, and of the number of neurones in the network. However, analog hCPGs are difficult to program not least because of their chaotic dynamics.

The challenge in engineering neural networks for medicine therefore lies in finding the network parameters (neuronal and synaptic conductances, channel gate and kinetic parameters) that enable the network to emulate the bCPG. For the simplest networks such parameters can be obtained by trial and error (Nogaret *et al.* 2013). For more complicated networks such as the mammalian respiratory CPG in Fig. 1, analytical techniques must be devised to

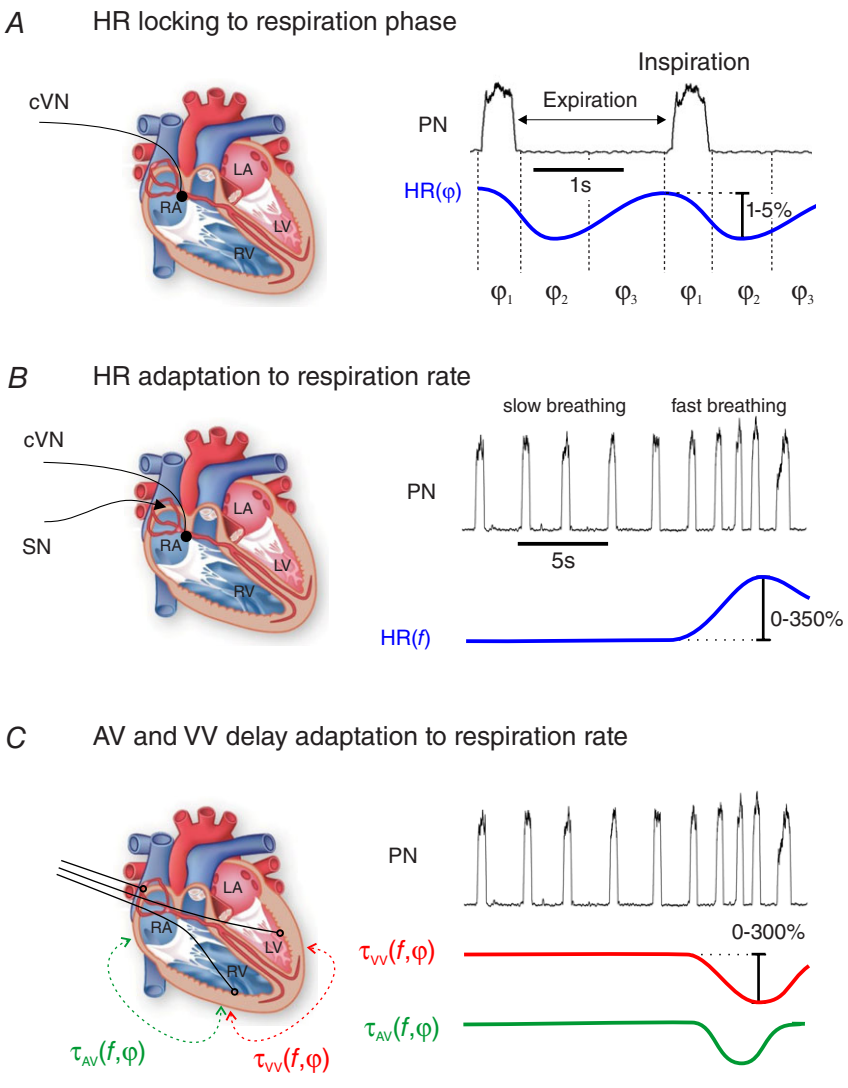

Figure 2. Challenges for hCPG pacemaker: adaptation of the heart to physiological feedback
A, vagus nerve (cVN) activity modulates heart rate across the respiratory cycle to produce respiratory sinus arrhythmia (RSA). The phase (and amplitude) of heart rate modulation could be shifted to any part of the respiratory cycle using a hCPG. RSA arises from the respiratory bCPG oscillator depicted in Fig. 1. *B*, heart rate (HR) correlates to the breathing rate to provide adaptation that is proportional to the increase in physical effort. This coupling is again based on brainstem circuitry (Fig. 1). *C*, the atrio-ventricular delay (τ_{AV}) and inter-ventricular delays (τ_{VV}) also adapt to heart rate. Beat-to-beat adaptation of these delays can be integrated in pacing by hCPGs. Abbreviations: AV, atrio-ventricular; *f*, frequency; PN, phrenic nerve (diaphragm); SN, sympathetic nerve; VV, inter-ventricular; ϕ, phase.

Table 1. Comparative merits of sCPGs and hCPGs

	Digital/software CPGs (sCPGs)		Analog/hardware CPGs (hCPGs)
Cons	Analog (in) → ADC → CPU → DAC → Analog (out)	Pros	Analog (in) → Analog neurons → Analog (out)
	Clocked digital electronics (CPU, ADC, DAC)		Asynchronous electronics capable of responding to stimuli in real time
	Slow numerical integration of real stimuli by Hodgkin–Huxley-like models		Instantaneous integration of stimuli
	Accuracy and speed of computation decreases when the network size increases		Accuracy and speed is independent of the size of the network
	Increasingly simple neuron approximations need to be considered as the network size increases to keep integration tractable		Realistic multichannel neurons can be integrated in networks of arbitrary size
	Hard to scale down		Easy to scale down to a few square millimetres (no ADC, DAC or CPU needed)
	Battery lifetime: 10 years		Battery lifetime >> patient life
Pros	Ease and flexibility of programming software	Cons	Complex programming of analog chips

Abbreviations: ADC, analog-to-digital converter; CPU, central processing unit; DAC, digital-to-analog converter.

generate these parameters automatically. Such techniques exist and have been developed in recent years to infer the parameters of systems of non-linear differential equations from the assimilation of electrophysiological data (Vanier & Bower, 1999; Jolivet *et al.* 2008; van Geit *et al.* 2008). It is therefore conceivable that if the activity of the PN, cVN and RVLM output neurons can be measured as a function of the electrical activity of the inputs, over a wide dynamic range of inputs (e.g. sensory feedback), a quantitative model of the respiratory CPG can be inferred and tested. Data assimilation methods have been used to build quantitative neuron models and this is what we describe in the next section.

Data assimilation – a novel automated method for building biologically accurate hCPGs

Mathematical tools that infer hidden parameters from experimental data are routinely used by medical practitioners when reconstructing internal organs with computer tomography. Inverse methods of this kind generally rely on the knowledge of a mathematical model (describing how X-rays interact with biological tissue) together with the measurement of a model variable (the intensity of scattered photons) to infer parameters inaccessible to the experimenter (the volume density of biological tissue). Inferring the internal parameters of neurons and networks, however, requires a more sophisticated algorithm that accounts for the non-linear nature of the models (Abarbanel, 1993). This methodology is based on Takens' embedding theorem that states that, under certain conditions, a non-linear system can be reconstructed from a sequence of observations of the state of a dynamic system (Takens, 1981). If the observation is the recording of a neuron membrane voltage, all internal parameters of the neuron (channel conductances, gate threshold voltages and kinetic parameters) may be inferred from the time series data as long as the current waveform stimulating the neuron is sufficiently varied to activate every time constant of the neuron. In this case, the inverse problem is fully constrained meaning that data assimilation yields a single value for each parameter of the model. These theoretical premises have in recent years spurred the search for parameter extraction methods capable of constructing quantitative neuron models by assimilation of the electrophysiological recordings of biological neurons (Vanier & Bower, 1999; Jolivet *et al.* 2008; van Geit *et al.* 2008). One approach started from semi-empirical Hodgkin–Huxley (Hodgkin & Huxley, 1952) models incorporating tabulated values for channel activation, inactivation and kinetic parameters and extracting the ion channel conductances. The problem of fitting these conductances to the electrophysiological data is essentially a linear one. Optimal conductances have been inferred from random parameter search (Tuckwell & Richter, 1978; Baldi *et al.* 1998; Goldman *et al.* 2001; Golowasch *et al.* 2002; Prinz *et al.* 2003; Huys *et al.* 2006; Lepora *et al.* 2012), evolutionary (Eiben & Smith, 2003; Hobbs & Hooper, 2008) and genetic algorithms (Achard & De Schutter, 2006; Druckmann *et al.* 2007; Kobayashi *et al.* 2009; Buhry *et al.* 2011; Hendrickson *et al.* 2011; Marasco *et al.* 2012;), gradient descent methods (Reid *et al.* 2007) and simulated annealing (Pospischil *et al.* 2008; Brookings *et al.* 2014). A second category of methods has been developed to infer the

non-linear parameters that control the time dependence and voltage dependence of channel conductances. Vavoulis *et al.* (2012) have extracted activation thresholds, time delays and ion channel conductances from *Lymnaea* motoneurons by performing time series analysis with Kitagawa's self-organising state space approach (Kitagawa, 1998) and the Kalman filter (Evensen, 2009). Kostuk *et al.* (2012) have demonstrated Monte Carlo path integral methods and Meliza *et al.* (Toth *et al.* 2012; Meliza *et al.* 2014) have modelled different populations of neurons from the songbird high vocal centre using interior point optimisation, a non-linear convex optimisation method (Gill *et al.* 2005; Wächter & Biegler, 2006). In a first step, the interior point filter automatically generates the parameters (i.e. membrane ionic conductances and their kinetics) that best synchronise the model to the experimental time series data (Fig. 3). In a second step, the completed neuron model is validated by comparing its response to any current waveform with experimental observation (Fig. 3). Neuron models built from data assimilation are remarkably accurate at making *quantitative predictions* of voltage oscillations elicited by arbitrary current waveforms (Meliza *et al.* 2014). This approach may now be extended to finding the parameters of networks to build hCPGs that accurately reproduce the behaviour of bCPGs.

Besides building predictive models, data assimilation offers a novel route for categorising neuron types and neuron functions based on the types of ion channels expressed in electrophysiological experiments. Provided the electrical parameters of ion channels can be extracted with sufficient accuracy, the method may identify subunits of ion channels based on the different conformal properties of their constituting proteins. Through assimilation of electrophysiological data, Meliza *et al.* (2014) have succeeded in identifying neurons projecting to different cortices of the songbird high vocal centre. Given the one-to-one correspondence between ion channels and genes (Warren *et al.* 2010; Lovell *et al.* 2013) data assimilation may also reveal the genes expressed in different categories of neurons. In summary, non-linear

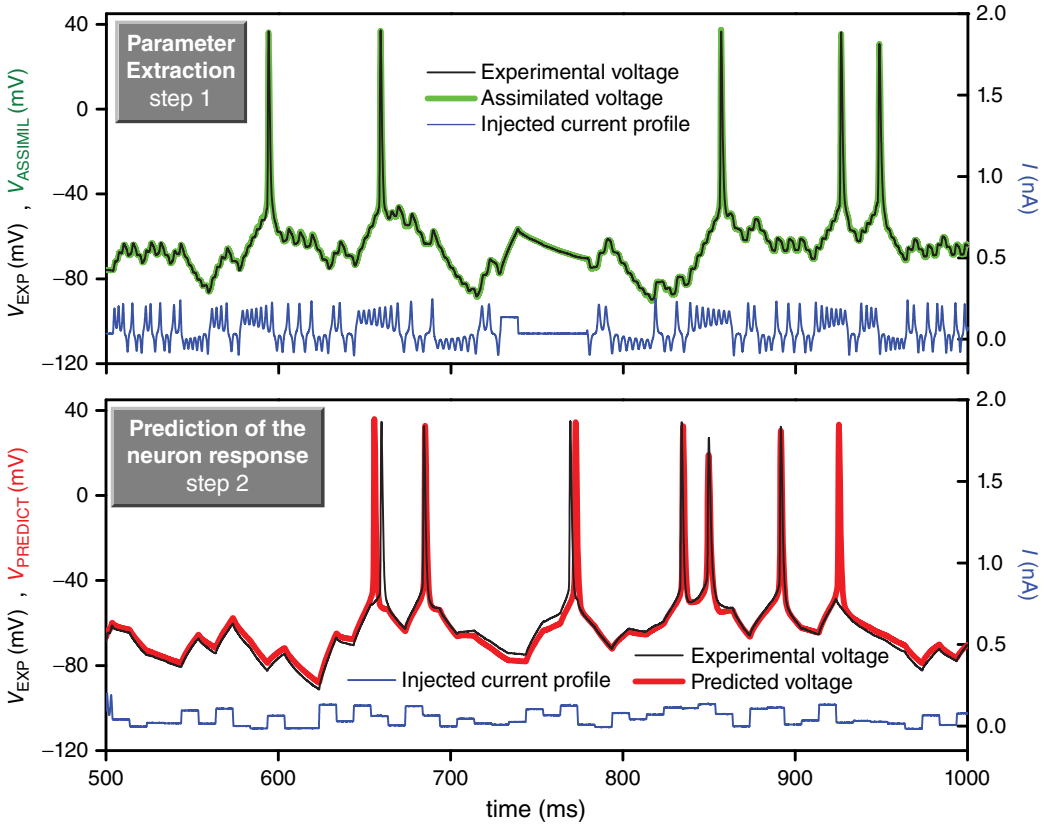

Figure 3. Construction of quantitative neuron models
Top, neuron parameters including the channel conductances, gate thresholds and kinetic parameters are extracted by assimilating electrophysiological recordings of interneurons of the songbird high vocal centre with interior point optimisation (green curve). The current protocol used to stimulate the neuron was generated by the chaotic Lorenz system. Bottom, predicted response made by integrating the experimental current protocol with the completed neuron model (red curve). The prediction is compared with the observed membrane voltage (black curve). This demonstrates the power of data assimilation for constructing predictive neuron (and network) models (after Meliza *et al.* 2014).

optimisation enables the construction of both neurons and neural network models that accurately emulate the response of biological CPGs. This recent work has removed a major obstacle to the engineering of efficient implantable hCPGs.

Proof of concept – artificially inducing respiratory sinus arrhythmia with two/three phase hCPG

The neuronal hCPG we have built is based on analog neurons which integrate the Hodgkin–Huxley equations in real time that is suitable for neuro-stimulation (Nogaret, 2012; Nogaret *et al.* 2013; Fig. 4). Our hCPG applies biphasic neuron bursts of controlled duration and timing to the vagus nerve, which we stimulate unidirectionally using selective anodal block clamp (Jones *et al.* 1995) to slow the heart rate. The stimulation can be phase tuned to target any point within the respiratory cycle with pulses of amplitude varying between 0 and 4.3 V (Fig. 5). Our hCPG synchronises heart rate modulation to the respiratory cycle by receiving signals from either the phrenic nerve or diaphragmatic electromyographic activity. Switching the timing of stimulation from inspiration to expiration affected the amplitude of heart rate modulation (Nogaret *et al.* 2013). The sensitivity of the heart rate response to stimulation in the different respiratory phases was found to vary (Nogaret *et al.* 2013) as shown in Fig. 5.

To test the adaptive ability and robustness of our hCPG, we changed respiratory rate and found that stimulation naturally synchronises to respiration even at high breathing rates (Fig. 6). This novel analog hCPG now provides us with the ability to induce RSA in rats and mimic some functional aspects generated within the cardio-respiratory networks of the ventrolateral medulla.

Future applications for bioelectronic medicine

Because of their stability and ability to integrate incoming physiological signals in real time, implantable analog hCPGs are likely to have numerous applications in both basic science and medical therapeutics where the endogenous rhythmic drive required by a biological system is suppressed or lost in disease. Table 1 emphasises the unique and potentially game changing advantages that analog hCPGs have over digital devices. Analog hCPGs will provide novel and more naturalistic ways to modulate body systems because of their adaptive characteristics allowing them to respond to changes in the environment. They can integrate incoming sensory information and provide an appropriate effective output dependent upon behavioural state.

An application we are exploring is cardiac pacemaking and the generation of RSA for improving cardiac function; the latter is based on a prediction we made using a new

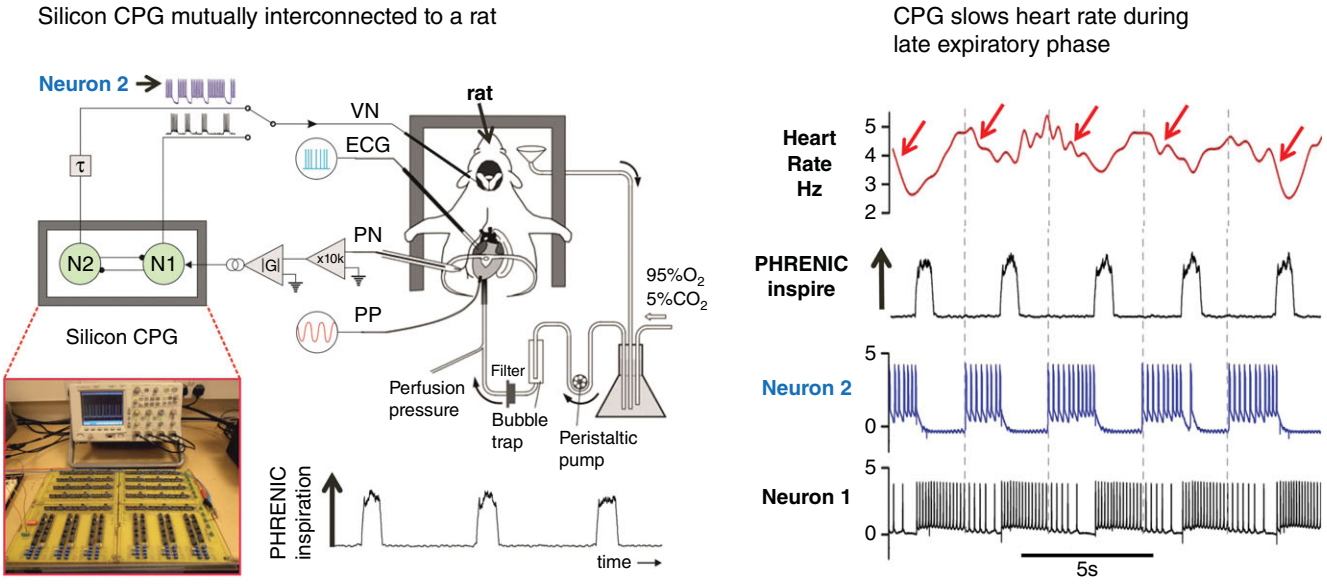

Figure 4. Slowing down of the heart rate in phase with respiration (*in vitro*)
The analog hCPG receives respiratory feedback from the phrenic nerve via filters, amplifiers and a rectifying transconductance amplifier |G| (conductance modulus). The r.m.s. amplitude of the raw phrenic signal (not show) is 60–100 μV. Using this information, the central pattern generator (CPG) stimulates the vagus nerve with neuron bursts in phase or out of phase with inspiration recorded from the phrenic nerve (PN). Neuron 2 is connected to the vagus nerve (VN) and slows down the heart rate by ~40% during the expiratory phase. Note: Neuron 1 is not connected to any output but is reciprocally connected to Neuron 2 to produce a two phase oscillator. Current work is to build a three neuron CPG to generate a three phase rhythm to emulate breathing *in vivo*. Abbreviation: ECG, electrocardiogram.

mathematical model (Ben-Tal *et al.* 2012, 2014). Current cardiac pacemakers are pre-set and although some have the ability to respond to acceleration or movement or body temperature this is limited and restrictive. The adaptive properties of hCPGs can be harnessed with data assimilation to provide heart rate adaptation to the rate of respiration and beat-to-beat adaptation of τ_{VV} and τ_{AV} delays in cardiac resynchronisation therapy, for example. This would, for the first time, provide physiological pacing and physiological gearing of the degree of exertion (as detected from changes in respiratory rate) with appropriate levels of cardiac pacing. This could include, for example, appropriate post-exercise pacing which is likely to be important in protecting a failing heart. Given the possibility for modulation of synaptic transmission at the level of the cardiac vagal ganglion McAllen *et al.* (2011),

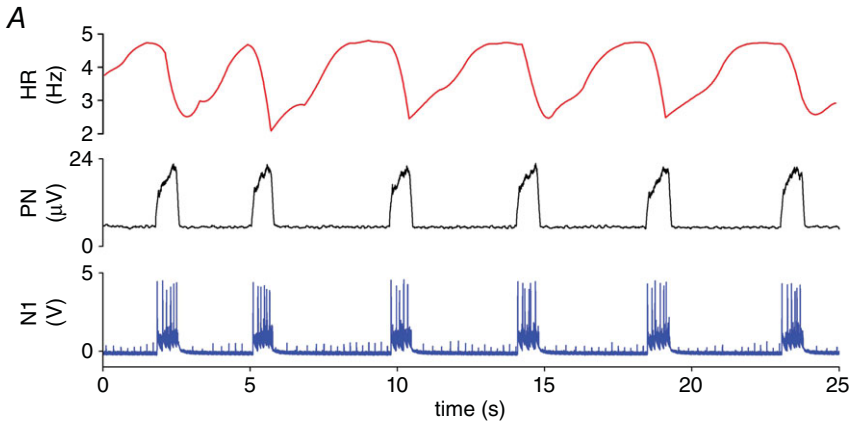

Figure 5. RSA induced by stimulating the vagus nerve in phase and out of phase with inspiration
The flexibility of the hCPG allows cardiac RSA pacing during either inspiration coincident with phrenic nerve activity (stimulation by Neuron 1 of hCPG -N1: 80 Hz; *A*) or during the latter part of the expiratory pause (stimulation by N2: 58 Hz; *B*). Abbreviations: HR, heart rate; PN, phrenic nerve; N1, Neuron 1 of hCPG; N2, Neuron 2 of hCPG (after Nogaret *et al.* 2013).

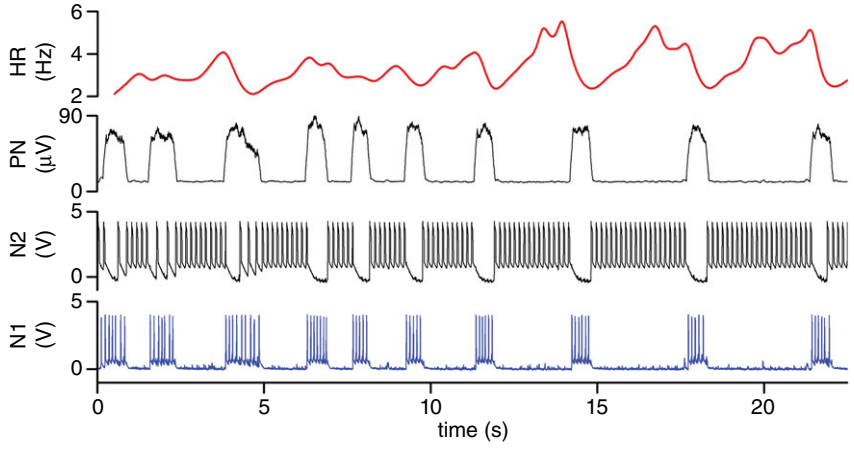

Figure 6. Adaptive response of the hCPG to variation in respiratory frequency
The dynamic response of the hCPG to a change in respiratory frequency is shown. Note how the hCPG tracks the change in respiratory rate precisely to maintain respiratory sinus arrhythmia. The reduction in RSA amplitude at the faster respiratory frequency reflects a lack of time for the full bradycardia to develop prior to the next inspiration. Abbreviations: HR, heart rate; PN, phrenic nerve; N1, Neuron 1 of hCPG; N2, Neuron 2 of hCPG (after Nogaret *et al.* 2013).

the data from Bibevski & Dunlap (1999) is most pertinent: synaptic transmission efficacy at the pre-ganglionic to post-ganglionic synapse (within the cardiac vagal ganglia) was depressed in a canine heart failure model. Clearly this would compromise the efficacy of such a device in heart failure, at least in dogs. However, recent data from the rat indicate no such loss of transganglionic transmission for controlling cardiac vagal activity up to 4 h post myocardial infarction (Passamani *et al.* 2014). Nevertheless, any application to a model (or human) in which transmission through the cardiac ganglion is suppressed in disease states can be circumvented by stimulating the SA node directly via the right atrium. This would also alleviate issues of vagus-induced negative ionotropism, which may compromise any beneficial effects of vagal-induced respiratory sinus arrhythmia pacing in the failing heart. Finally, other applications might include generation of respiratory and locomotor rhythmicity following spinal cord injury but this all remains a challenge for the future.

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Additional information

Competing interests

None declared.

Authors contributions

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